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<b>(54) Title:</b> COMPOSITION AND METHOD FOR PRESERVING PROGENITOR CELLS  <b>(57) Abstract</b>  The invention relates to a protein material which is effective to preserve progenitor cells, such as hematopoietic progenitor cells. The protein has an amino acid sequence comprising AQSLSFSFTKFD (SEQ ID NO.1) and a molecular weight of about 12-20 kD, or has an amino acid sequence comprising VVAVEFD (SEQ ID NO.3) and a molecular weight of about 15-20 kD. Heterodimers of the protein are described, and multimers thereof. Methods of using the protein of the invention for preserving progenitor cells <i>in vitro</i> , <i>ex vivo</i> , and <i>in vivo</i> are also described. The invention, therefore, includes methods such as myeloablation therapies for cancer treatment wherein myeloid reconstitution is facilitated by means of the specified protein. Other therapeutic utilities are also enabled through the invention, for example, expanding progenitor cell populations <i>ex vivo</i> to increase changes of engraftation, improving conditions for transporting and storing progenitor cells, and facilitated gene therapy to treat and cure a broad range of life-threatening hematologic diseases.		

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## **COMPOSITION AND METHOD FOR PRESERVING PROGENITOR CELLS**

### **BACKGROUND OF THE INVENTION**

5 The invention relates to an agent and method for use in connection with progenitor cells. More specifically, the invention relates to a protein capable of preserving progenitor cells and a method of using the protein for maintaining and preserving progenitor cells.

Each day the bone marrow generates and releases into the circulation several billion fully-differentiated, functional blood cells. Production of these cells derives from a small stock of quiescent progenitor cells (including the most primitive stem cells and other less primitive but still immature progenitors) by a process called hematopoiesis (Zipori 1992). The most  
10 primitive stem cells have the capacity to generate  $>10^{13}$  cells containing all blood lineages (Turhan et al. 1989). The production of such a large number of cells is achieved by extensive proliferation coupled with successive differentiation steps leading to a balanced production of mature cells. Progenitor cells progressively lose their capacity to generate multiple cells  
15 lineages and eventually produce cells of one or two cell lineages.

Soluble regulators and cell-cell interactions mediate differentiation directions of immature progenitors through a tightly-controlled but inadequately understood process. Several of the body's soluble factors have been isolated and characterized both in culture and in animals (see, e.g., Ogawa (1993) and references therein). Regulators such as the colony  
20 stimulating factors (e.g., IL3, GM-CSF, G-CSF, M-CSF) not only induce proliferation and differentiation of progenitors capable of producing cells of either multiple cell lineages (IL3 and GM-CSF) or single cell lineages (G-CSF and M-CSF), but also maintain viability of their respective progenitors. Other regulators such as interleukin-1 (IL1), the kit ligand (KL), and thrombopoietin (Borge et al. 1996) increase viability of multipotential progenitors in addition  
25 to other functions. No known cytokines alone or in combination can maintain viability of primitive progenitors in liquid culture without stromal support beyond a few days.

Regulation of primitive stem cells appears to differ from that of immature, multilineage progenitors. Stem cells are primarily quiescent and do not appear to respond immediately to regulators that induce proliferation and differentiation. Maintenance of these cells in the body  
30 is mediated via cell-cell interactions and soluble regulators. Maintenance of quiescent stem cells *in vitro* has been achieved by culturing cells on adherent stromal layers with soluble

regulators such as IL3, IL6, KL and LIF (Young et al. 1996). Recently, the addition of FL to this complex culture has been found to extend maintenance of quiescent stem cells from a few weeks to three months (Shah et al. 1996). Establishing stromal cells cultures is not easily applicable to clinical settings.

5           Lectins, defined as carbohydrate-binding proteins other than antibodies or enzymes, (Baronedes 1988), are widespread among plants, prokaryotes, and eukaryotes. Each lectin recognizes a specific carbohydrate moiety, and forms a non-covalent bond with the carbohydrate through a stereochemical fit of complementary molecules (e.g., hydrophobic pocket). Carbohydrates are widely present on cell surfaces (in the forms of glycoproteins, glycolipids, and polysaccharides), and appear to mediate cell-cell contacts including cell  
10           recognition (Sharon et al. 1989). Abnormal glycosylation patterns are associated with disease by causing alterations in a protein's conformation, stability, or protease resistance (Dwek 1995).

          Gowda et al. (1994) describe the isolation of a mannose-glucose-specific lectin from  
15           the hyacinth bean (*Dolichos lab lab*). Purification and sequencing of this lectin is said to indicate that the protein includes two nonidentical subunits. The Gowda et al. publication describes evolutionary relationships of the lectin to other lectins, but does not ascribe any function to the protein beyond saccharide-binding in the plant source.

          Cell agglutinating properties of certain plant lectins have been known for over 100  
20           years. Certain lectins have been used as tools in immunology laboratories as potent, specific activators of T lymphocytes (phytohemagglutinin (PHA) and concanavalin A (ConA)) and B lymphocytes (pokeweed mitogen (PWM)) for over 30 years (Sharon et al. 1989). Some lectins have also been used to isolate hematopoietic progenitors for over 15 years (Gabius 1994a). Large numbers of cancer patients in Europe have received mistletoe lectin (*Viscum*  
25           *album*) intravenously as a candidate cancer therapy without major complications (Gabius 1994b). Whether these plant lectins act on mammalian cells via *de novo* means, or simply mimic their functional mammalian homologs is not yet known. No lectin has yet been successfully developed as a human therapeutic.

          In view of the above considerations, it is clear that regulation of the hematopoietic  
30           process remains incompletely understood. Most soluble regulators identified, such as the colony stimulating factors and interleukins, induce proliferation and differentiation of

progenitors cells in culture and their levels in the blood circulation increase during times of hematopoietic stress (e.g., blood loss, infection). For example, U.S. Patent No. 4,808,611 describes a method of using IL1 and a colony stimulating factor to induce proliferation and differentiation of hemopoietic stem cells. Some soluble regulators, such as IL1, IL6, KL, FL, and Tpo, appear to provide increase viability of stem cells without directly affecting proliferation and differentiation. But no known soluble regulators, alone or in combination, have yet been reported that enable maintenance and amplification of stem cells populations *in vitro* without stromal cells. As a consequence, numerous potential therapeutic approaches to diseases such as cancer and genetic blood diseases remain unavailable.

Accordingly, it is one of the purposes of this invention to overcome the above limitations in methods of regulating hematopoietic processes, by providing a factor and method of protecting, maintaining, and expanding hematopoietic progenitor cell populations. It is another purpose of the invention to provide means for protecting the integrity of the hematopoietic processes *in vivo* as an adjunct to therapeutic treatments related to cancer and other diseases which can otherwise adversely impact upon the hematopoietic system.

### SUMMARY OF THE INVENTION

It has now been discovered that these and other objectives can be achieved by the present invention, which provides a protein which preserves progenitor cells and a method of using the protein. The protein has an amino acid sequence comprising AQSLSFSFTKFD (SEQ ID NO:1) and a molecular weight of about 12-20 kD, or has an amino acid sequence comprising VVAVEFD (SEQ ID NO:3) and a molecular weight of about 15-20 kD. The method of preserving progenitor cells comprises contacting progenitor cells with a protein, having an amino acid sequence comprising AQSLSFSFTKFD (SEQ ID NO:1) and a molecular weight of about 12-20 kD, or having an amino acid sequence comprising VVAVEFD (SEQ ID NO:3) and a molecular weight of about 15-20 kD, in an amount sufficient to preserve the progenitor cells.

In one embodiment, the invention includes a method of treating a mammal in need of hematopoietic therapy. Here the method comprises:

- a) obtaining a tissue sample from the mammal, the tissue sample comprising hematopoietic progenitor cells;

- b) culturing the progenitor cells in the presence of a protein which preserves the progenitor cells, to provide cultured cells enriched in the progenitor cells, wherein the protein has an amino acid sequence comprising AQSLSFSFTKFD (SEQ ID NO:1) and a molecular weight of about 12-20 kD, or has an amino acid sequence comprising VVAVEFD (SEQ ID NO:3) and a molecular weight of about 15-20 kD;
- c) subjecting the mammal to conditions sufficient to effect myeloablation; and
- d) administering the cultured cells to the mammal following the myeloablation to reconstitute the hematopoietic system of the mammal.

In an alternative embodiment, the invention includes a method of enriching progenitor cells. The method comprises culturing progenitor cells in a progenitor-preserving amount of a protein having an amino acid sequence comprising VVAVEFD (SEQ ID NO:3) and a molecular weight of about 15-20 kD, or having an amino acid sequence comprising AQSLSFSFTKFD (SEQ ID NO:1) and a molecular weight of about 12-20 kD, wherein the protein specifically preserves the progenitor cells, and wherein the culturing is performed under conditions permitting preservation of progenitor cells while permitting the number of differentiated cells to decrease.

In still another embodiment, the invention includes a method of improving hematopoietic competence in a mammal. In this case, the method comprises:

- a) culturing a tissue sample comprising hematopoietic progenitor cells in a growth medium containing a protein having an amino acid sequence comprising AQSLSFSFTKFD (SEQ ID NO:1) and a molecular weight of about 12-20 kD, or having an amino acid sequence comprising VVAVEFD (SEQ ID NO:3) and a molecular weight of about 15-20 kD, in an amount sufficient to preserve the progenitor cells and to provide cultured cells enriched in the progenitor cells; and
- b) transfusing the enriched cultured cells to the mammal to provide progenitor cells for generating blood cellular components in the mammal.

In yet another embodiment, the invention further includes a method of transfecting an exogenous DNA sequence into somatic cells, which includes the improvement comprising transfecting progenitor cells selectively preserved by a protein having an amino acid sequence comprising AQSLSFSFTKFD (SEQ ID NO:1) and a molecular weight of about 12-20 kD, or

having an amino acid sequence comprising VVAVEFD (SEQ ID NO:3) and a molecular weight of about 15-20 kD.

In a further embodiment, the invention includes a composition for maintaining viability of progenitor cells *ex vivo*, comprising a cell growth medium and a protein which preserves progenitor cells, wherein the protein has an amino acid sequence comprising  
5 AQSLSFSFTKFD (SEQ ID NO:1) and a molecular weight of about 12-20 kD, or has an amino acid sequence comprising VVAVEFD (SEQ ID NO:3) and a molecular weight of about 15-20 kD.

Moreover, in yet another embodiment, the invention includes, a method for preserving progenitor cells in a mammal. The method of this embodiment comprises:  
10

a) administering to the mammal a protein which specifically preserves progenitor cells, the protein having an amino acid sequence comprising AQSLSFSFTKFD (SEQ ID NO:1) and a molecular weight of about 12-20 kD, or having an amino acid sequence comprising VVAVEFD (SEQ ID NO:3) and a molecular weight of about 15-20 kD, in an amount  
15 sufficient to preserve progenitor cells of the mammal in a substantially non-proliferative state;

b) exposing the mammal to myeloablative conditions sufficient to effect ablation of proliferating myeloid cells but sparing non-proliferating progenitor cells; and

c) following the exposing, reestablishing proliferation or differentiation of the preserved progenitor cells.  
20

These and other advantages of the present invention will be appreciated from the detailed description and examples which are set forth herein. The detailed description and examples enhance the understanding of the invention, but are not intended to limit the scope of the invention.

### BRIEF DESCRIPTION OF THE DRAWINGS

Preferred embodiments of the invention have been chosen for purposes of illustration and description, but are not intended in any way to restrict the scope of the invention. The preferred embodiments of certain aspects of the invention are shown in the accompanying drawings, wherein:  
25

Figure 1 shows an electrophoretic separation of protein illustrating the alpha and beta subunit structure of the pylartin protein obtained from hyacinth beans.  
30

Figure 2A is a graph of the effect of the protein of the invention on FLK2-transfected 3T3 cells in the presence of IL1; Figure 2B is a graph of the effect of the protein of the invention on FLK2-transfected 3T3 cells in the absence of IL1; Figure 2C is a graph of the effect of the protein of the invention on untransfected 3T3 cells in the presence of IL1; Figure 2D is a graph of the effect of the protein of the invention on untransfected 3T3 cells in the absence of IL1.

Figure 3 is a series of histograms comparing the protein of the invention, obtained from red kidney beans, and from hyacinth beans, against culture medium control, showing that the protein of the invention specifically preserves murine CD34<sup>+</sup> cells in liquid culture, with and without a cocktail of cytokines including mIL1, mIL3, and mKL.

Figure 4 is a series of histograms comparing the protein of the invention, obtained from red kidney beans or hyacinth beans, against culture medium control, showing that the protein of the invention specifically preserves murine progenitor cells in a methylcellulose colony assay, with and without a cocktail of cytokines including mIL1, mIL3, and mKL.

Figure 5 is a series of histograms showing the results of flow cytometric study of six different cell surface markers showing that the protein of the invention specifically preserves primitive murine progenitors.

Figure 6A is a set of histograms comparing the protein of the invention against culture medium control, showing that the protein of the invention specifically preserves CD34<sup>+</sup> human cord blood cells in liquid culture for six days; Figure 6B is a complementary set of histograms showing that the protein of the invention specifically preserves CD34<sup>+</sup> human cord blood cells in liquid culture for 17 days.

Figure 7 is a set of two histograms comparing the protein of the invention against a control, illustrating that the pylartin protein, either alone or in combination with IL1-a or FL preserves or increases the number of human progenitor cells in a methylcellulose colony assay.

Figures 8A-8C are a set of histograms showing that the protein of the invention, and not FL, preserves primitive hematopoietic progenitor cells in liquid culture after 15 and 21 days.

Figures 9A and 9B are a set of histograms showing that the protein of the invention preserves hematopoietic progenitors in a dose-responsive manner.



Figure 10 is a pair of histograms, comparing the protein of the invention against medium control, illustrating that the pylartin protein preserves progenitors by preventing IL3-induced differentiation.

#### DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

5       The present invention is directed to a protein which preserves progenitor cells, and a method for use of the protein in preserving and maintaining progenitor cells. By "protein" is meant any isolated natural or synthetic oligo- or polypeptide comprising a sequence of amino acid residues linked by peptide bonds.

10       As used herein, "progenitor cell" refers to any somatic cell which has the capacity to generate fully differentiated, functional progeny by differentiation and proliferation. Progenitor cells include progenitors from any tissue or organ system, including, but not limited to, blood, nerve, muscle, skin, gut, bone, kidney, liver, pancreas, thymus, and the like. Progenitor cells are distinguished from "differentiated cells," which are defined as those cells which may or may not have the capacity to proliferate, i.e., self-replicate, but which are unable  
15       to undergo further differentiation to a different cell type under normal physiological conditions. Moreover, progenitor cells are further distinguished from abnormal cells such as cancer cells, especially leukemia cells, which proliferate (self-replicate) but which generally do not further differentiate, despite appearing to be immature or undifferentiated.

20       Progenitor cells include all the cells in a lineage of differentiation and proliferation prior to the most differentiated or the fully mature cell. Thus, for example, progenitors include the skin progenitor in the mature individual, which is capable of differentiation to only one type of cell, but which is itself not fully mature or fully differentiated. Production of mature, functional blood cells results from proliferation and differentiation of "unipotential progenitors," i.e., those progenitors which have the capacity to make only one type of one  
25       type of blood cell. For red blood cell production, a progenitor called a "CFU-E" (colony forming unit-erythroid) has the capacity to generate two to 32 progeny cells.

30       Various other hematopoietic progenitors have been characterized. For example, hematopoietic progenitor cells include those cells which are capable of successive cycles of differentiating and proliferating to yield up to eight different mature hematopoietic cell lineages. At the most primitive or undifferentiated end of the hematopoietic spectrum,

hematopoietic progenitor cells include the hematopoietic "stem cells." These rare cells, which represent 1 in 10,000 to 1 in 100,000 of cells in the bone marrow, each have the capacity to generate  $>10^{13}$  mature blood cells of all lineages and are responsible for sustaining blood cell production over the life of an animal. They reside in the marrow primarily in a quiescent state and may form identical daughter cells through a process called self-renewal. Accordingly, such an uncommitted progenitor can be described as being "totipotent," i.e., both necessary and sufficient for generating all types of mature blood cells. Progenitor cells which retain a capacity to generate all blood cell lineages but which can not self-renew are termed "pluripotent." Cells which can produce some but not all blood lineages and can not self-renew are termed "multipotent."

The protein of the invention is useful to preserve any of these progenitor cells, including unipotent progenitor cells, pluripotent progenitor cells, and/or totipotent progenitor cells. The protein has demonstrated particular efficacy in preserving hematopoietic progenitor cells, however, the protein can be used to preserve progenitor cells of other tissues such as those mentioned above. Because of its properties in controlling the hematopoietic process, the protein of the invention is also referred to herein as "pylartin" or "the pylartin protein." However, it should be recognized that the protein is also useful in the preservation and maintenance of progenitor cells in non-hematopoietic tissues.

The pylartin protein is especially useful in preserving hematopoietic progenitors in mammals such as humans, mice, rats, etc. In the human, primitive mature hematopoietic progenitor cells can be identified as belonging to a class of cells defined by their expression of a cell surface antigen designated CD34. These cells may be referred to as CD34<sup>+</sup> cells. In the mouse, hematopoietic progenitor cells may be referred to as Sca<sup>+</sup>Lin<sup>-</sup> cells, reflecting their cell surface antigen signature. Other mammalian species exhibit similar signature properties identifying hematopoietic progenitor cells. Hematopoietic progenitors can also be identified by their expression of the FLK2/FLT3 receptor.

Human hematopoietic progenitor cells which express the CD34 antigen and/or the FLK2 receptor are referred to herein as "primitive progenitor cells." By contrast, hematopoietic cells which do not express either the CD34 antigen or the FLK2 receptor are referred to as "mature progenitor cells."

Generally, the pylartin protein is effective to preserve progenitor cells which express the CD34 antigen, or the FLK2 receptor. The progenitor cells can include cells modified to express the CD34 antigen or FLK2 receptors on their surface. In a preferred case, the protein has significant binding affinity for FLK2 receptor on the cells, wherein binding of the protein with the FLK2 receptor mediates the inhibition of differentiation of the cells.

The pylartin protein mediates a "preservation" of progenitor cells. By this is meant that the peptide either inhibits differentiation of the progenitor cells, or induces their proliferation without differentiation. In particular, by inhibiting differentiation processes, it is to be understood that the peptide significantly lowers the rate at which cells differentiate, and it may in fact completely stop these processes by possibly maintaining progenitor cells in a quiescent or G0 state of the cell cycle. However, the protein does so without killing the cells in significant numbers. In this sense, pylartin is significantly distinguished from factors which inhibit or interfere with cellular processes (e.g., DNA replication, protein synthesis), and which thereby induce significant cell mortality.

The pylartin protein of the invention can be obtained from a natural source, namely being derived from a legume. A legume is a fruit or seed (e.g., a pea or bean) of a leguminous plant, which are plants from a family (*Leguminosae*) of dicotyledonous herbs, shrubs, and trees bearing (nitrogen-fixing bacteria) nodules on their roots. More specifically, the pylartin protein can be obtained from members of the tribe *Phaseoleae*. In particular, the protein can be obtained from hyacinth beans (*Dolichos lab lab*), kidney beans, e.g., red kidney beans (*Phaseolus vulgaris*), or the black-eyed pea (*Vigna senensis*). In its native form obtained from such natural sources, the protein appears to be a mannose/glucose-specific legume lectin.

In one embodiment, the pylartin protein comprises a peptide chain designated  $\beta$  (beta), which comprises the sequence AQSLSFSFTKFD (SEQ ID NO:1). Preferably, the  $\beta$  peptide comprises the sequence AQSLSFSFTKFDLD (SEQ ID NO:2). The  $\beta$  peptide has a molecular weight in the range of about 12-20 kD, typically about 13 kD.

In an alternative embodiment, the pylartin protein comprises a peptide chain designated  $\alpha$  (alpha), which comprises the sequence VVAVEFD (SEQ ID NO:3). Preferably, the  $\alpha$  peptide comprises the sequence TDSYVVAVEFD (SEQ ID NO:4). The  $\alpha$  peptide has a molecular weight in the range of about 15-20 kD, typically about 16 kD.

In a further alternative embodiment a protein comprises a peptide chain having the sequence VVAVEDFD (SEQ ID NO:5) or TDSYVVAVEDFD (SEQ ID NO:6). The protein of this embodiment has properties and functions that are substantially comparable to those described elsewhere herein.

5 In another embodiment, the protein can include monomeric, dimeric and multimeric proteins, in which one, two, or more than two peptide chains, respectively, occur together as subunits of a single proteinaceous substance, whether of natural or synthetic origin. The subunits of such dimers and multimers may be the same or they may be different. A highly preferred dimer according to the invention is a heterodimer comprising one alpha peptide and  
10 one beta peptide ( $\alpha\beta$ ).

The pylartin heterodimers ( $\alpha\beta$ ) may aggregate, presumably by non-covalent interactions, to form a tetramer ( $\alpha\beta$ )<sub>2</sub> with a mass of about 60 kD. The tetramer appears to be the most common naturally occurring form of the protein, but other multimers, e.g., concatamers of the heterodimer, i.e., ( $\alpha\beta$ )<sub>n</sub> wherein n is a positive integer larger than 2, may  
15 be observable by SDS-PAGE, in mass increments of about 25-30 kD. Accordingly, the invention encompasses all of these configurations. In its mature form as an  $\alpha_2\beta_2$  tetramer, the pylartin protein retains biological activity at pH 2-10; multiple freeze-thaw cycles; at 60°C for ten minutes; and at 4°C for several months.

Certain molecular features of the pylartin protein indicate that the protein is related to  
20 known plant lectins, specifically to lectins derived from legumes. For example, the N-terminal region of the  $\alpha$  peptide includes a plant lectin consensus sequence, i.e., residues 5-12 of SEQ ID NO:3. Also, the pylartin protein can occur as a tetramer, a structure common to many lectins.

Other features of the pylartin protein are remarkable and unexpected. For example,  
25 the protein of the invention has been obtained, as described below, from the red kidney bean, which is known to produce another lectin (i.e., phytohemagglutinin) in much larger quantities. That a plant would express two such distinctly different lectins is unexpected. It is known that certain plant proteins, such as the aforementioned phytohemagglutinin, have physiological effects in mammals. Nonetheless, it is not expected that any protein of indeterminate function  
30 from one taxonomic kingdom would also act as a specific regulator of progenitor cells in another kingdom. Surely such a phenomenon is unexpected and unexplained. In addition,

whereas certain eukaryotic proteins are identified as playing a role in developmental control (e.g., Tan-1 for hematopoietic lineages), no comparable role has yet been defined for plant lectins.

As a result of the present invention, numerous utilities become technically feasible.

5 The method of the invention can include contacting the progenitor cells with the pylartin protein *in vitro*, *ex vivo*, or *in vivo*. For example, the pylartin protein finds a utility, *inter alia*, in that it enables *ex vivo* maintenance of hematopoietic progenitor cells isolated from either normal or malignant (e.g., leukemic) bone marrow. Accordingly, the protein can be employed in the culture of mononuclear cells derived from a source of such cells, for example, from bone  
10 marrow, umbilical cord blood, placental blood, or peripheral blood. Alternatively, the pylartin protein can be used in conjunction with growth factors such as colony stimulating factors (CSFs) (e.g., IL3, GM-CSF, G-CSF, M-CSF), interleukins (e.g., IL1 through IL15) and KL *in vitro* to selectively induce proliferation and terminal differentiation of mature progenitors while leaving a highly enriched population of primitive progenitors. U.S. Patent Nos.  
15 5,472,867 and 5,186,931 describe representative methods of using CSFs and interleukins (ILs) to expand progenitor cell populations in the contexts of, respectively, cancer chemotherapy and bone marrow transplants. In a preferred case according to the present invention, the method further includes contacting the progenitor cells with FLK2 ligand in an amount sufficient to selectively expand the number of progenitor cells without inducing differentiation  
20 thereof.

The pylartin protein also enhances survival of progenitor cells when cultured *in vitro*. For example, a process of *in vitro* selection can be used which involves using the protein to preserve progenitor cells in a substantially quiescent state in culture, while using a cytotoxic agent which exhibits selective toxicity for proliferating cells, e.g., to kill cells passing through  
25 cell cycle ("cycling cells"). Suitable cytotoxic agents include, for example, compounds such as adriamycin, cyclophosphamide, taxol or other taxane, cisplatin, or 5-fluorouracil. The method is useful to preserve quiescent progenitor cells. The method is effective even when the progenitor cells are substantially free of stromal cells, cells which are considered to normally be necessary for progenitor cell maintenance and proper hematopoietic reconstitution.  
30 Pylartin improves the ability to functionally select stem cells either alone or with other factors.

Such functional selection methods, include the method reported by Berardi et al. (1995) where selection is made using a combination of KL and IL3 with 5-FU.

5       *Ex vivo* purging protocols can be used to selectively eliminate neoplastic cells by targeting the elimination of actively cycling cells. By preserving progenitor cells in a quiescent state, the protein of the invention preserves normal progenitor cells, while the cycling cells are killed. Once the progenitors cells have been purged of malignant cycling cells, they can be returned to the patient, and permitted to resume normal proliferation and differentiation. In one especially useful scenario, the pylartin protein allows for functional selection of normal progenitor cells from a leukemic bone marrow. Such functional selection and purification of  
10       primitive stem cells can also be used to enable allogeneic transplant procedures.

      The pylartin protein also enables *ex vivo* manipulation of hematopoietic progenitor cells for use in gene therapy by maintaining cells in liquid culture. For example, by preserving hematopoietic progenitor cells in culture for more than two weeks, the protein enables increased targeting efficiency by viral vectors that enter non-replicating cells (e.g., vectors  
15       such as adeno-associated viruses), and provides longer periods for the evaluation of the resultant cell populations to determine efficiency of transfection. Thus, in another embodiment, the method can be used in conjunction with methods of transfecting an exogenous DNA sequence into somatic cells. The method can then include transfecting progenitor cells selectively preserved by the pylartin protein.

20       The invention also has utility in conjunction with therapies, e.g., cancer therapies, which employ irradiation. Specifically, because the pylartin protein maintains progenitor cells in a quiescent state, administration of the pylartin protein to a mammalian subject *in vivo* allows the use of increased levels of total body irradiation to eliminate neoplastic cells, while leaving quiescent cells relatively unaffected. The protein can be employed in conjunction with  
25       other cytoprotective substances such as IL-1 to obtain an enhanced or complementary effect.

      Thus, the method can involve treating a mammalian subject in need of hematopoietic therapy. For example, a tissue sample including hematopoietic progenitor cells can be obtained from the subject. Then the tissue sample can be cultured *ex vivo* in the presence of the pylartin protein to preserve the progenitors, while allowing cycling cells to proliferate,  
30       differentiate and die. The cultured cells become highly enriched in the primitive progenitor cells. Meanwhile, the mammal can be subjected to conditions sufficient to effect

myeloablation, e.g., bone marrow irradiation, whole body irradiation, or chemically-induced myeloablation. Finally, the progenitor-enriched cultured cells can be administered to the mammal following the myeloablation to reconstitute the hematopoietic system of the mammal. While described here as an autologous procedure, the skilled practitioner will recognize that  
5 the method can be readily adapted to transplant of progenitor-enriched cells from one individual to another.

The pylartin protein also improves hematopoietic competence in a mammal, i.e., the mammal's ability to generate functional mature blood elements. For example, a tissue sample comprising hematopoietic progenitor cells can be cultured in a growth medium containing the  
10 pylartin protein in an amount sufficient to preserve the progenitor cells and to provide cultured cells enriched in the progenitor cells. Then, the enriched cultured cells are transfused to the mammal to provide progenitor cells for generating blood cellular components in the mammal. This method can further comprise ablating hematopoietic tissues in the mammal prior to the transfusing. In addition, the method can use a tissue sample comprising peripheral blood,  
15 umbilical cord blood, placental blood, or bone marrow. Preferably, the tissue sample is autologous to the mammal. The tissue sample can also be at least substantially free of stromal cells.

The invention further includes a composition for maintaining viability of progenitor cells *ex vivo* or *in vitro*. The composition comprises a culture medium suitable for growth and  
20 maintenance of mammalian cells in culture, along with an amount of the pylartin protein sufficient to preserve progenitor cells as described herein. Virtually any cell or tissue culture medium can be modified for the preservation of progenitors in this way. Ready-to-use receptacles, e.g., blood bags, microtiter plates, and tissue culture flasks, and the like, can also be provided with the pylartin protein (with or without culture medium or other active  
25 components) to promote culture of progenitor cells.

Also, the invention includes a method for preserving progenitor cells in a mammal *in vivo*. In this approach, the method comprises administering to the mammal the pylartin protein in an amount sufficient to preserve progenitor cells of the mammal in a substantially non-proliferative state. The mammal is then exposed to myeloablative conditions sufficient to  
30 effect ablation of proliferating myeloid cells but sparing non-proliferating progenitor cells. Following the ablative treatment, proliferation or differentiation of the preserved progenitor

cells is reestablished, usually by administering to the mammal a cytokine in an amount sufficient to improve the viability of the progenitor cells. Preferred viability-improving cytokines include, for example, IL1, IL3, IL6, IL11, KL, or a combination thereof. More preferably, the reestablishing comprises administering to the mammal a

5 proliferation-stimulating amount of the FLK2 ligand. According to this method, the pylartin protein can be used to enhance autologous bone marrow transplantation techniques in which lethal doses of radiation and/or chemotherapy are followed by reinfusion of stored marrow.

An effective amount of pylartin protein can be administered to a mammal by any convenient approach, such as parenteral routes, e.g., intravenous injection, or preferably by

10 enteral routes. Oral administration routes are expected to be useful since lectins typically resist oral/gastric degradation, and can exhibit substantial bioavailability by this approach. The skilled artisan recognizes the utility and limitations of various methods of administration and can adjust dosage (posology) accordingly.

Other therapeutic utilities also present themselves to the skilled practitioner as being

15 enabled by the invention. Such other utilities include, for example, expanding progenitor cell populations *ex vivo* to increase chances of engraftation, improving conditions for transporting and storing progenitor cells, and removing a fundamental barrier to enable gene therapy to treat and cure a broad range of life-threatening hematologic diseases such as sickle cell anemia and thalassemia.

The protein of the invention can also be used as a specific probe for the identification

20 or localization of progenitor cells. Since the protein binds specifically to primitive progenitor cells, a composition including the protein linked to a detectable moiety such as a fluorescent marker can be used to specifically label and identify progenitor cells. Thus, cell sorting to isolate progenitor cells can be performed, as can histologic localization of progenitor cells in

25 tissues. The skilled artisan can select the type or marker moiety to be employed according to the task to be performed, since numerous methods of labeling proteins are known in the art.

The following examples are provided to assist in a further understanding of the invention. The particular materials and conditions employed are intended to be further illustrative of the invention and are not limiting upon the reasonable scope thereof.



**EXAMPLE 1****Isolation of the Pylartin Protein from Kidney Beans and Hyacinth Beans**

Two hundred fifty grams (250 g) of kidney beans obtained from a commercial source (Stokes Seed Company, Buffalo, New York or W. Atlee Burpee & Co, Warminster, Pennsylvania) was pulverized in a coffee grinder. (This method can also be used to isolate material from hyacinth beans or other beans containing an active substance in accordance with the invention.) The bean powder was added to 1 L of an alkaline lectin binding buffer (LBB; e.g., 50 mM Tris/HCl, pH 7.0, containing metal cations: 1 mM each of  $MgCl_2$  and  $CaCl_2$ ) and incubated with constant mixing overnight at 4°C. The particulate matter was pelleted by centrifugation at 10,000 x g for 20 min and the supernatant was retained.

The protein binds specifically to mannose, enabling a single step purification of the protein. To remove contaminants that could interfere with mannose-agarose affinity chromatography, the bean supernatant was exposed to ammonium sulfate precipitation using 60% (0.6 g/mL). The precipitate was resuspended, and dialyzed overnight at 4°C against LBB, to provide the starting material for affinity chromatography. The dialyzed ammonium sulfate fraction was incubated with mannose covalently coupled to Sepharose 4B (Sigma Chemical Co., St. Louis, MO) overnight on a rocker at 4°C. The gel was then washed extensively with LBB and pelleted by gravity sedimentation. Lectins were eluted with 250 mM methyl  $\alpha$ -D-methylpyranoside (Sigma Chemical Co., St. Louis, MO) in LBB for two days on a rocker at 4°C, pelleted by density sedimentation, and the lectin-containing supernatant aspirated. The supernatant containing the pylartin protein was dialyzed overnight against LBB to remove sugar, and the resulting dialysate was sterile filtered, and stored at -20°C.

Figure 1 shows SDS-PAGE analysis of purified pylartin protein isolated from hyacinth beans, and purified by mannose-agarose. Pylartin samples and molecular markers (Integrated Separation Systems, Natwick, MA) were analyzed under reducing conditions on a 10-27% gradient polyacrylamide gels (Integrated Separation Systems, Natwick, MA) and visualized by Coomassie Brilliant Blue staining (Sigma Chemical Co., St. Louis, MO). Molecular weights are indicated on left side of the gel; alpha and beta subunits are indicated for each on the right

side of each gel and numbers indicate isoforms of subunits. Four isoforms of the alpha subunit and one of the beta subunit of pylartin were detected in the hyacinth bean.

## EXAMPLE 2

### FLK2 Receptor 3T3 Assay Specifically Quantitates Biological Activity of the Pylartin Protein

5           The pylartin protein interacts with the mammalian FLK2 tyrosine kinase receptor. A specific and quantitative biological assay using NIH 3T3 fibroblasts transfected either with a chimeric receptor consisting of the extracellular portion of the murine FLK2 receptor combined with the intracellular portion of the human Fms receptor (Lemischka et al., unpublished) or with the full length human receptor (Small et al. 1994) can be used to evaluate  
10       lectin biological activity during purification. Serial two-fold dilutions of lectin samples across rows of a 96 well plate allowed for greater than a thousand-fold range to access FLK2 3T3 biological activity. The assay requires either the murine or human FLK2/FLT3 ligand (FL) (Lyman et al. 1993; Hannum et al. 1994) or the pylartin protein to rescue FLK2-transfected cells from death.

15           Specifically, remove 3T3 cells from stock culture plates by washing cells cultured in tissue culture plates (Becton Dickinson Labware, Lincoln Park, New Jersey) twice in Hank's buffered saline (HBSS; Gibco Laboratories, Grand Island, New York) followed by addition of non-enzymatic cell dissociation buffer (Gibco) for 15 minutes at room temperature, and subsequently wash cells in medium. Culture FLK2 3T3 cells at a final concentration of 3,000  
20       cells per well in a volume of 100  $\mu$ L of serum-defined medium containing 10% AIMV (Gibco) and 90% Dulbecco's modification of Eagle's medium (DMEM; Gibco) in 96 well plates. Under these assay conditions, cells die after two to four days of culture in a humidified incubator at 37°C and 5% CO<sub>2</sub> unless exogenously added ligand rescues cells from death. Each 96 well plate contains calf serum, which stimulates all 3T3 cells, as a positive control and  
25       medium only as a negative control. Full-length Fms-transfected 3T3 cells (biological response shown in Tessler et al. 1994) serve as receptor-transfected control target cells, and parent 3T3 cells serve as untransfected control cells. Proliferation and cell survival is quantitated by addition of XTT (Diagnostic Chemicals Ltd, Charlottetown, Prince Edward Island, Canada), a tetraformazan salt cleaved by actively respiring cells (Mosmann 1983), and quantitated  
30       spectrophotometrically using a Vmax kinetic plate reader (Molecular Devices Corp., Mountain

View, CA), and recorded as either relative activity (units/mL) or as specific activity (units/mg). One unit of biological activity is defined as the reciprocal dilution at which half-maximal stimulation of cells is detected. Figures 2A-2D show that pylartin isolated from red kidney beans specifically stimulates FLK2 3T3 cells in an IL1-dependent manner. Pylartin biological was tested over a 1,000-fold range on FLK2/Fms 3T3 cells (Figures 2A and 2B) and parent untransfected 3T3 cells (Figures 2C and 2D) in the absence (Figures 2A and 2C) and presence (Figures 2B and 2D) of 10 ng/mL human recombinant interleukin 1-alpha (IL1- $\alpha$ ) (BioSource International, Camarillo, CA) and quantitated by XTT. The pylartin protein stimulated proliferation of FLK2/Fms 3T3 cells only in the presence of IL1, and did not stimulate proliferation of untransfected 3T3 cells regardless of whether IL1 was present or not.

Examples 3-10, below, show that the protein of the invention preserves progenitors cells *in vitro*. Pylartin isolated from red kidney beans and hyacinth beans specifically preserves progenitors derived from murine fetal liver cells and human cord blood cells in liquid culture.

### 15 **EXAMPLE 3**

#### The Pylartin Protein Preserves Human and Murine Hematopoietic Progenitor Cells *In Vitro*

Hematopoietic progenitor cells range in frequency from about 1:10,000 to about 1:100,000 of mononuclear cells in human bone marrow, umbilical cord blood, and under certain conditions, peripheral blood. Enrichment of progenitor cells for *in vitro* culture involves isolating mononuclear cells from a sample of whole blood or blood marrow by density separation. Specifically, the sample is gently layered over Ficoll-Paque Plus (Pharmacia Biotech, Piscataway, NJ) in a 50 mL conical tube (Becton Dickinson, Franklin Lakes, NJ) and the samples are processed according to the manufacturer's directions.

Enrichment of stem cells greater than a hundred-fold from mononuclear cell preparations can be achieved using antibody-labeled magnetic bead technology. Two commercial methods (DYNABEADS, available from Dynal Corp., Lake Success, NY, and MINIMACS multiparameter sorting, Miltenyi Biotec, Auburn, CA), rely on positive selection of CD34<sup>+</sup> cells. Another method (STEMSEP CD34<sup>+</sup>/CD38<sup>-</sup>, available from Stem Cell Technologies, Inc., Vancouver, BC) isolates stem cells by negative selection (glycophorin A,

CD14, CD3, CD16, CD2, CD66b, CD56, CD36, CD24, CD45RA, CD19, CD38), resulting in cells with the surface phenotype of CD34<sup>+</sup>, CD36<sup>-</sup>, CD38<sup>-</sup>, CD45RA<sup>-</sup>.

The pylartin protein is used to preserve the isolated progenitor cells, either within a population of mononuclear cells or in enriched CD34<sup>+</sup> cells, in liquid culture containing serum-defined medium (AIMV-V from Gibco, Grand Island, NY; BIT9500 from Stem Cell Technologies, Vancouver, BC; or X-VIVO 10 from BioWhittaker, Walkersville, MD) in an incubator maintained at 37°C and flushed with 5% CO<sub>2</sub>, for two to four weeks. The resultant cell population is characterized by flow cytometry, and hematopoietic colony assays are performed to enumerate and evaluate the functional status of the progenitor cells.

Human hematopoietic stem cells express the cell surface antigen phenotypic profile of CD34<sup>++</sup> CD38<sup>-</sup> Thy<sup>+</sup>, but do not express antigens for mature blood cell lineages (CD3, CD19, etc.) (Peltzer et al. 1996). Specifically, cells are incubated with fluorescent-labeled antibodies (e.g., Coulter Corp., Hialeah, Florida) following manufacturer's directions and are evaluated for marker expression by flow cytometry (e.g., Epics Elite, Coulter Corp.).

Hematopoietic colony assays indirectly assess the frequency and primitive status of progenitors in a population of cells. Cells are cultured in semi-solid methylcellulose containing growth factors for optimal stimulation of cell proliferation and differentiation (Stem Cell Technologies, Vancouver, BC).

#### EXAMPLE 4

##### Pylartin Preserves Murine Fetal Liver CD34<sup>+</sup> Cells in Culture

Figure 3 shows that purified pylartin isolated from red kidney beans (Pylartin-RK) and hyacinth beans (Pylartin-H), either alone or together with increasing concentrations of a cytokine cocktail (mIL1, mIL3, mKL), preserves murine fetal liver CD34<sup>+</sup> cells for six days in liquid culture medium containing 10% calf serum in DMEM. After fifteen days the cultures were harvested and cells replated in a methylcellulose colony assay to assess frequency of functional progenitors. NT indicates not tested. Mononuclear cells cultured in the presence or absence of pylartin with either no exogenous cytokines or a cocktail of early-acting recombinant murine cytokines (mIL1, mIL3, mKL) (BioSource International, Camarillo, CA) all at either 5 ng/mL or 50 ng/mL. Flow cytometric analysis of remaining viable cells shows

that the proportion of CD34<sup>+</sup> cells in culture wells containing pylartin increases in a dose-dependent manner of early acting cytokine cocktail is present but not in medium control.

## EXAMPLE 5

### Pylartin Preserves Murine Hematopoietic Progenitors in Culture

5           The pylartin protein, either alone or in combination with either IL1 or FL, preserves functional hematopoietic progenitors for at least two weeks in culture. Specifically, Figure 4 shows the preservative effect of purified pylartin on murine hematopoietic progenitors cultured fifteen days in liquid culture medium containing 10% calf serum in DMEM. Evaluation of functional progenitors was tested by harvested cells in liquid culture and  
10           replated in methylcellulose colony assays (Stem Cell Technologies, Vancouver, British Columbia) according to the manufacturer's instructions. Colonies derived from individual progenitors were scored after two weeks; results were normalized for frequency of colonies per 100,000 cells initial seeded. Each of the pylartin samples preserved progenitors in the absence of the exogenous cytokine cocktail (left bars each group); variable differences were  
15           observed for cytokine cocktail-containing pylartin samples.

## EXAMPLE 6

### Flow Cytometric Analysis of Cell Surface Markers on Cells Cultured with Pylartin Protein

          The type of cells generated in methylcellulose colony assays in cultures containing 50 ng/mL of the cytokine cocktail (Figure 4) was determined by flow cytometry. These data  
20           are summarized in Figure 5. Sca<sup>+</sup> cells were greatly increased in pylartin-containing cultures; other cell surface markers were not particularly affected. The Sca antigen is expressed on murine stem cells and other mature cells including T cells. In this experiment, T cells (CD3) were not increased. Figure 5 shows a flow cytometric analysis of the cell surface phenotype of cells harvested from cells cultured in 50 ng/mL of early-acting recombinant murine cytokines  
25           (mIL1, mIL3, mKL) (BioSource International, Camarillo, CA), in the presence and absence of pylartin samples, prior to colony assay (far right bars from Fig. 4). Pylartin-RK generated 14.5-fold more Sca-1 cells than medium control. Sca-1, or Ly6, is an antigen associated with primitive murine progenitors and also mature blood cells including T cells, etc. (Spangrude et al. 1991). No differences were observed in the numbers of T cells (CD3), dendritic cells

(CD11b), or cells expressing the Fc-gamma receptor (e.g., granulocytes and monocytes), CD38, or the transferrin receptor (CD71) (antibodies obtained from Pharmigen, CA).

#### EXAMPLE 7

##### The Pylartin Protein Preserves Human CD34<sup>+</sup> Cells in Culture

5 Pylartin also preserves human primitive progenitors. Figures 6A and 6B show that pylartin, either alone or in combination with early-acting human cytokines (rhIL1- $\alpha$ , rhIL3, rhFL, rhKL), specifically preserves CD34<sup>+</sup> human cord blood cells for up to two weeks. The purified pylartin isolated from hyacinth beans (Pylartin-H) specifically preserves human cord blood cells that express the CD34 antigen for seventeen days (Fig. 6A) and six days (Fig. 6B)  
10 in liquid culture containing a serum-defined medium (BIT9500, Stem Cell Technologies, Vancouver, British Columbia). Pylartin is effective to preserve CD34<sup>+</sup> cells either with or without added exogenous cytokines.

#### EXAMPLE 8

##### The Pylartin Protein Preserves Human Progenitors in Liquid Culture

15 The pylartin protein, either alone or in combination with either IL1 or FL, preserves functional progenitors for at least two weeks in liquid culture. Figure 7 shows in this experiment the combination of pylartin and FL actually increases the number of progenitors in liquid culture under selected assay conditions. The number of colonies derived from functional progenitors is several times greater where pylartin is present (left panel) than in  
20 medium control (right panel), with or without exogenous IL1 or FL.

The nature of hematopoietic progenitors preserved by pylartin in liquid culture changes over time. Cord blood mononuclear cells (mnc) were cultured in X-VIVO 10 at a concentration of 800,000 cells in 4 mL for 6, 15, and 21 days with either pylartin, FL, pylartin + FL, or medium control. Functional progenitors were assessed by replating cells in  
25 methylcellulose colony assays and scoring colonies after an additional 16 days. Four types of colonies were recorded: "Blast" refers to small, dispersed colonies consisting of undifferentiated cells which may represent very primitive stem cells; "Mix" refers to colonies consisting of differentiated myeloid and erythroid cells formed by multilineage progenitors; "Myeloid" refers to colonies consisting exclusively of differentiated myeloid cells; "Erythroid"

refers to colonies consisting exclusively of differentiated erythroblasts or normoblasts formed by primitive progenitors called "BFU-E" (burst-forming unit erythroid).

Colonies formed in all liquid culture wells after six days of culture (Figure 8A). The solid line in Figure 8A indicates too many colonies to accurately score, which in this experiment was greater than 1,500 colonies per 800,000 mnc.

After 15 days of culture pylartin but not FL preserves relatively mature progenitors of myeloid or erythroid lineages (Figure 8B). "Blast"-like cells and myeloid-committed progenitors appear in methylcellulose colony assay plates from cord blood mononuclear cells preserved in liquid culture for 21 days (Figure 8C). The relatively high frequencies of progenitors cultured for 15 and 21 days indicate not only preservation of progenitors but also an increase (self-renewal) of primitive progenitors.

#### EXAMPLE 9

##### Pylartin Preserves Progenitor Cells in Culture in a Dose-Responsive Manner

Pylartin acts in a dose-responsive manner to preserve human cord blood progenitors. Figures 9A-9B show results from culturing cord blood mononuclear samples from two donors (Figures 9A and 9B, respectively) using the method described in Example 8. In this case, cultures were maintained for 12 days over a 100-fold concentration range of pylartin. Although the response differed between the two donors—both in composition of colony types and progenitor frequency—the specific activity of DLL, at 20,000 units/mg, is approximately the same in each case. TMTC indicates too many colonies to count.

#### EXAMPLE 10

##### Pylartin Preserves Progenitors in Culture by Preventing Differentiation into Mature Blood Cells

Figure 10 shows that pylartin, obtained in this case from hyacinth beans, prevents IL3-induced production of cells expressing the Fc-gamma receptor after six days in liquid culture. IL3-containing cultures contained approximately 80% CD32<sup>+</sup> cells, while pylartin-containing cultures contained <10% CD32<sup>+</sup> cells. Cells that express FcγII and FCγIII receptors in mice include natural killer cells, monocytes, macrophages, granulocytes, mast cells, as well as fetal thymocytes (Unkeless 1979).

The data presented in the above Examples demonstrate that pylartin preserves primitive hematopoietic progenitors, either alone or in combination with other cytokines, and prevents IL3-induced differentiation in liquid culture. Indeed, the protein by itself is more effective in preserving progenitors than FL even when the latter is supplemented with stromal cells. While no data have yet been obtained to test whether the peptide and stromal cells are cultured together, applicants believe that a potentiation of the preservative effect of the peptide would be maintained. Nonetheless, it is apparent that the protein of the invention is effective in preserving primitive hematopoietic progenitors in cell culture. This implies that the protein is effective for use in clinical methods involving explantation of progenitors, culture and preservation of the progenitors *ex vivo*, and subsequent reimplantation (or transplantation) of the preserved progenitors.

Thus, while there have been described what are presently believed to be the preferred embodiments of the present invention, those skilled in the art will realize that other and further embodiments can be made without departing from the spirit of the invention, and it is intended to include all such further modifications and changes as come within the true scope of the claims set forth herein.



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## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

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- (ii) TITLE OF INVENTION: COMPOSITION AND METHOD FOR  
PRESERVING PROGENITOR CELLS
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 12 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Ala Gln Ser Leu Ser Phe Ser Phe Thr Lys Phe Asp  
1 5 10

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Ala Gln Ser Leu Ser Phe Ser Phe Thr Lys Phe Asp Leu Asp  
1 5 10

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Val Val Ala Val Glu Phe Asp  
1 5

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 11 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 12 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEO ID NO:6:

27

**WHAT IS CLAIMED IS:**

1. A protein that preserves progenitor cells, wherein the protein has an amino acid sequence comprising AQSLFSFTKFD (SEQ ID NO:1) and a molecular weight of about 12-20 kD, or has an amino acid sequence comprising VVAVEFD (SEQ ID NO:3) and a molecular weight of about 15-20 kD.

2. A protein according to Claim 1, wherein the protein comprises a heterodimer comprising an alpha polypeptide having an amino acid sequence comprising VVAVEFD (SEQ ID NO:3) and a molecular weight of about 15-20 kD, and a beta polypeptide having an amino acid sequence comprising AQSLFSFTKFD (SEQ ID NO:1) and a molecular weight of about 12-20 kD.

3. A protein according to Claim 1, wherein the protein has an amino acid sequence comprising TDSYVVAVEFD (SEQ ID NO:4).

4. A protein according to Claim 1, wherein the protein has an amino acid sequence comprising AQSLFSFTKFDLD (SEQ ID NO:2)

5. A protein according to Claim 2, wherein the protein comprises two or more heterodimers.

6. A protein according to Claim 5, wherein the protein is tetrameric, comprising two identical heterodimers.

7. A protein according to Claim 1, wherein the protein is a mannose/glucose-specific legume lectin.

8. A protein according to Claim 7, wherein the protein is obtained from a legume from the tribe Phaseoleae.

9. A protein according to Claim 8, wherein the protein is obtained from kidney beans, hyacinth beans, or black-eyed peas.

10. A protein according to Claim 1, wherein the progenitor cells are at least unipotent progenitor cells.

11. A protein according to Claim 1, wherein the progenitor cells are pluripotent progenitor cells.

12. A protein according to Claim 1, wherein the progenitor cells are totipotent progenitor cells.

13. A protein according to Claim 1, wherein the progenitor cells comprise hematopoietic progenitor cells.

14. A protein according to Claim 1, wherein the progenitor cells comprise nerve, muscle, skin, gut, bone, kidney, liver, pancreas, or thymus progenitor cells.

15. A protein according to Claim 11, wherein the progenitor cells express the CD34 antigen.

16. A protein according to Claim 11, wherein the progenitor cells express the flk2 receptor.

17. A protein according to Claim 16, wherein the progenitor cells comprise cells modified to express flk2 receptors on their surface.

18. A protein according to Claim 16, wherein the protein has significant binding affinity for flk2 receptor on the cells, wherein binding of the protein with the flk2 receptor mediates the inhibition of differentiation of the cells.

19. A method for preserving progenitor cells, comprising contacting progenitor cells with a protein, having an amino acid sequence comprising AQSLSFSFTKFD (SEQ ID NO:1) and a molecular weight of about 12-20 kD, or having an amino acid sequence comprising VVAVEFD (SEQ ID NO:3) and a molecular weight of about 15-20 kD, in an amount  
5 sufficient to preserve the progenitor cells.
20. A method according to Claim 19, wherein the protein has an amino acid sequence comprising AQSLSFSFTKFDLD (SEQ ID NO:2)
21. A method according to Claim 19, wherein the protein has an amino acid sequence comprising TDSYVVAVEFD (SEQ ID NO:4).
22. A method according to Claim 19, wherein the protein is obtained from a legume from the tribe Phaseoleae.
23. A method according to Claim 22, wherein the protein is obtained from kidney beans, hyacinth beans, or black-eyed peas.
24. A method according to Claim 19, wherein the progenitor cells comprise hematopoietic progenitor cells.
25. A method according to Claim 24, wherein the progenitor cells comprise human cells which express the CD34 antigen and/or the flk2 receptor.
26. A method according to Claim 24, wherein the progenitor cells comprise murine cells which express the Sca antigen but which do not express mature blood cell lineage antigens.
27. A method according to Claim 19, wherein the method comprises contacting the progenitor cells with the protein in vitro, ex vivo, or in vivo.



28. A method according to Claim 19, wherein the method further comprises contacting the progenitor cells with flk2 ligand in an amount sufficient to selectively expand the number of progenitor cells without inducing differentiation thereof.

29. A method of treating a mammal in need of hematopoietic therapy, comprising:

a) obtaining a tissue sample from the mammal, the tissue sample comprising hematopoietic progenitor cells;

5 b) culturing the progenitor cells in the presence of a protein which preserves the progenitor cells, to provide cultured cells enriched in the progenitor cells, wherein the protein has an amino acid sequence comprising AQSLFSFTKFD (SEQ ID NO:1) and a molecular weight of about 12-20 kD, or has an amino acid sequence comprising VVAVEFD (SEQ ID NO:3) and a molecular weight of about 15-20 kD;

c) subjecting the mammal to conditions sufficient to effect myeloablation; and

10 d) administering the cultured cells to the mammal following the myeloablation to reconstitute the hematopoietic system of the mammal.

30. A method according to Claim 29, wherein the myeloablation conditions comprise bone marrow irradiation, whole body irradiation, or chemically-induced myeloablation.

31. A method of enriching progenitor cells, comprising culturing progenitor cells in a progenitor-preserving amount of a protein having an amino acid sequence comprising VVAVEFD (SEQ ID NO:3) and a molecular weight of about 15-20 kD, or having an amino acid sequence comprising AQSLFSFTKFD (SEQ ID NO:1) and a molecular weight of about 12-20 kD, wherein the protein specifically preserves the progenitor cells, and wherein the culturing is performed under conditions permitting preservation of progenitor cells while permitting the number of differentiated cells to decrease.

32. A method according to Claim 31, wherein the progenitor cells include primitive progenitor cells.

33. A method according to Claim 31, wherein the progenitor cells include mature progenitor cells.

34. A method according to Claim 31, wherein the progenitor cells are at least substantially free of stromal cells.

35. A method according to Claim 31, wherein said culturing conditions include culturing in a medium containing a cytotoxic agent which exhibits selective toxicity for proliferating cells.

36. A method according to Claim 35, wherein the cytotoxic agent is adriamycin, cyclophosphamide, taxol or other taxane, cisplatin, or 5-fluorouracil.

37. A method of improving hematopoietic competence in a mammal, comprising:

5 a) culturing a tissue sample comprising hematopoietic progenitor cells in a growth medium containing a protein having an amino acid sequence comprising AQSLSFSFTKFD (SEQ ID NO:1) and a molecular weight of about 12-20 kD, or having an amino acid sequence comprising VVAVEFD (SEQ ID NO:3) and a molecular weight of about 15-20 kD, in an amount sufficient to preserve the progenitor cells and to provide cultured cells enriched in the progenitor cells; and

b) transfusing the enriched cultured cells to the mammal to provide progenitor cells for generating blood cellular components in the mammal.

38. A method according to Claim 37, wherein the tissue sample comprises peripheral blood, umbilical cord blood, placental blood, or bone marrow.

39. A method according to Claim 38, wherein the tissue sample is autologous to the mammal.

40. A method according to Claim 37, wherein said tissue sample is at least substantially free of stromal cells.

41. A method according to Claim 37, further comprising ablating hematopoietic tissues in the mammal prior to the transfusing.

42. In a method of transfecting an exogenous DNA sequence into somatic cells, the improvement comprising transfecting progenitor cells selectively preserved by a protein having an amino acid sequence comprising AQSLSFSFTKFD (SEQ ID NO:1) and a molecular weight of about 12-20 kD, or having an amino acid sequence comprising  
5 VVAVEFD (SEQ ID NO:3) and a molecular weight of about 15-20 kD .

43. A composition for maintaining viability of progenitor cells ex vivo, comprising a cell growth medium and a protein which preserves progenitor cells, wherein the protein has an amino acid sequence comprising AQSLSFSFTKFD (SEQ ID NO:1) and a molecular weight of about 12-20 kD, or has an amino acid sequence comprising VVAVEFD  
5 (SEQ ID NO:3) and a molecular weight of about 15-20 kD.

44. A method for preserving progenitor cells in a mammal, comprising:

- a) administering to said mammal a protein which specifically preserves progenitor cells, said protein having an amino acid sequence comprising AQSLSFSFTKFD (SEQ ID NO:1) and a molecular weight of about 12-20 kD, or having an amino acid sequence  
5 comprising VVAVEFD (SEQ ID NO:3) and a molecular weight of about 15-20 kD, in an amount sufficient to preserve progenitor cells of said mammal in a substantially non-proliferative state;
- b) exposing the mammal to myeloablative conditions sufficient to effect ablation of proliferating myeloid cells but sparing non-proliferating progenitor cells; and
- 10 c) following said exposing, reestablishing proliferation or differentiation of the preserved progenitor cells.

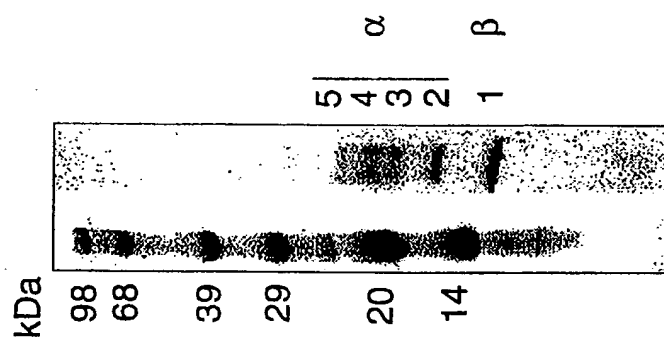
45. A method according to Claim 44, wherein said reestablishing comprises administering to said mammal a cytokine in an amount sufficient to improve the viability of the progenitor cells

46. A method according to Claim 45, wherein said viability-improving cytokine is IL-1, IL-3, IL-6, IL-11, KL, or a combination thereof.

47. A method according to Claim 44, wherein said reestablishing comprises administering to said mammal a proliferation-stimulating amount of the flk2 ligand.

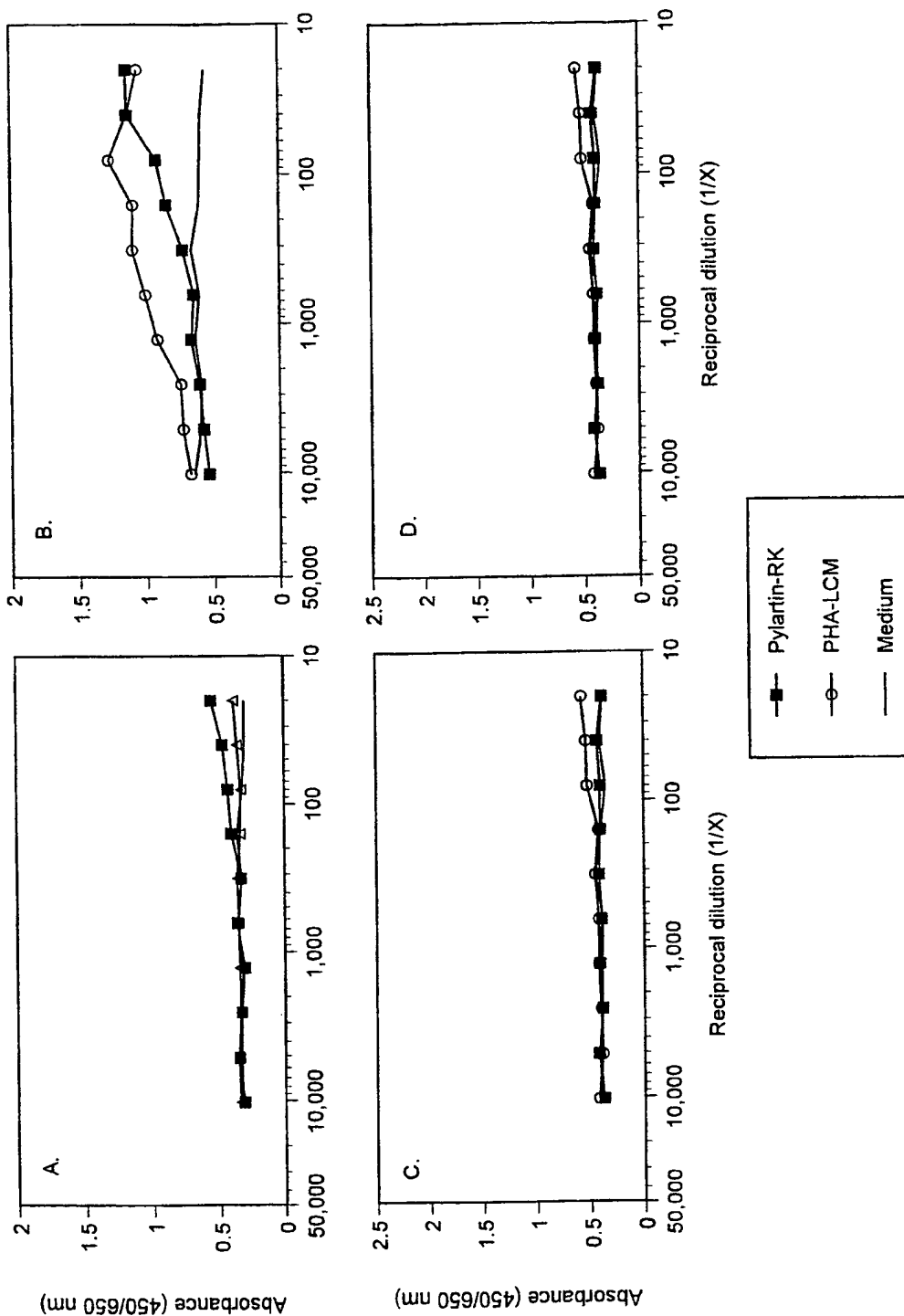
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Figure 1



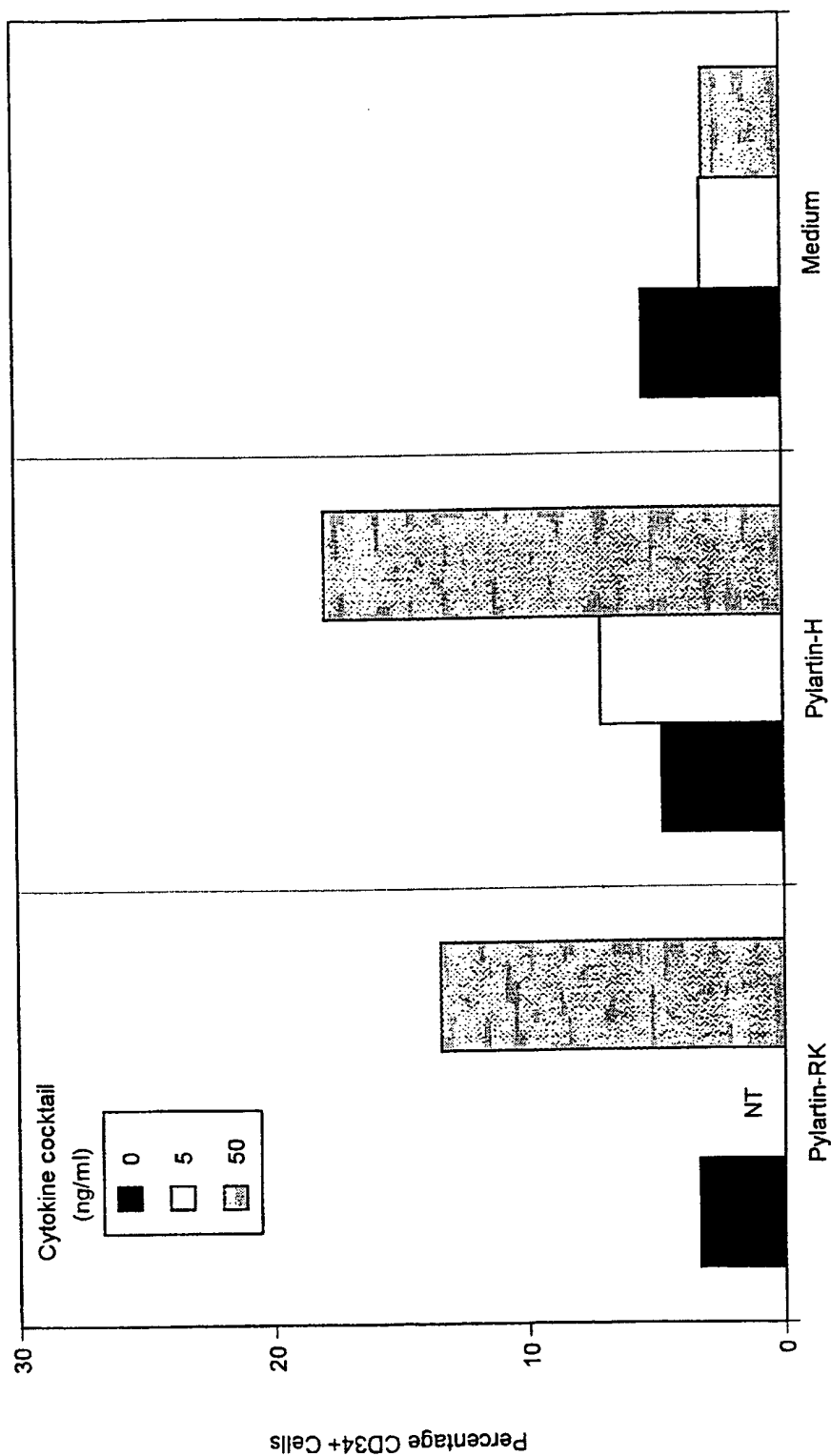
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Figures 2A - 2D



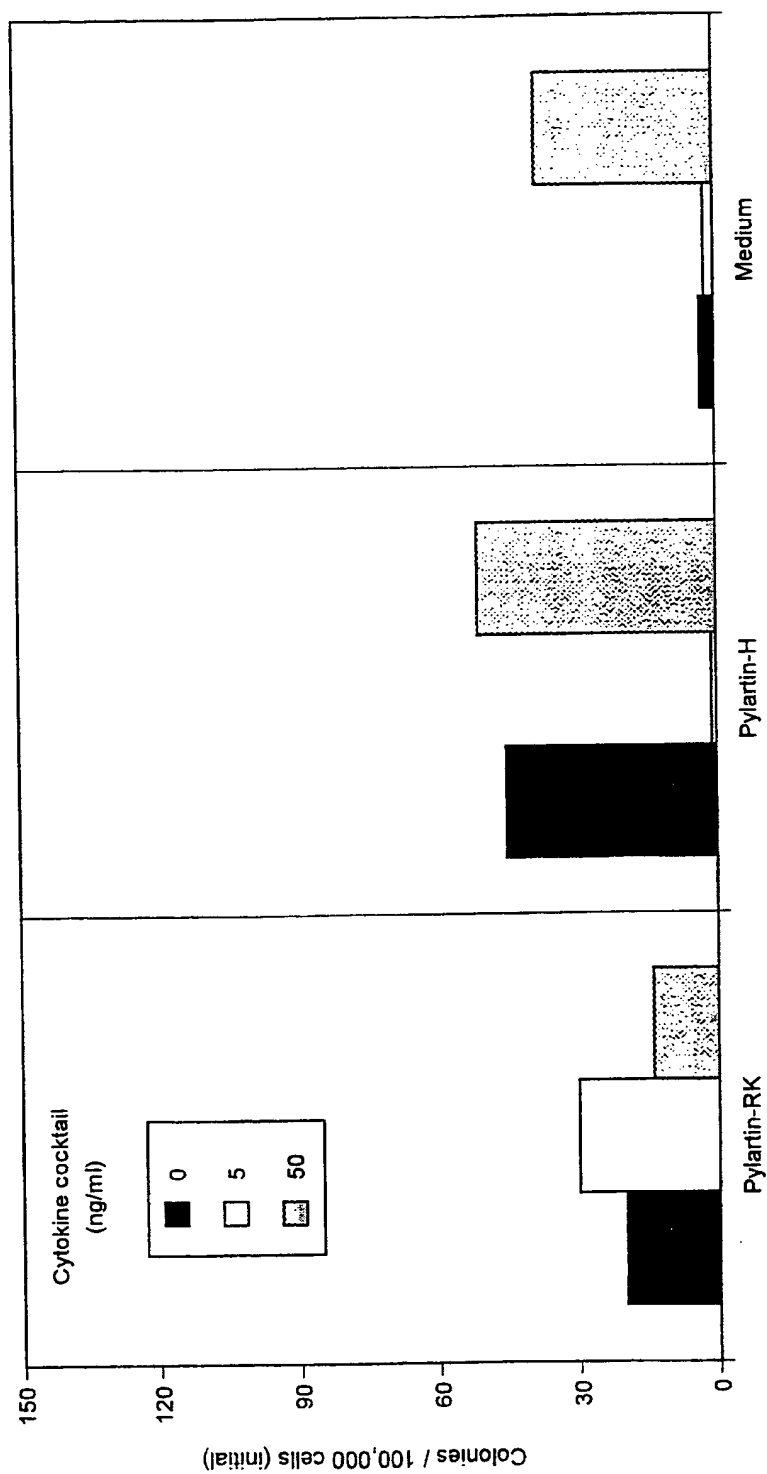
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Figure 3



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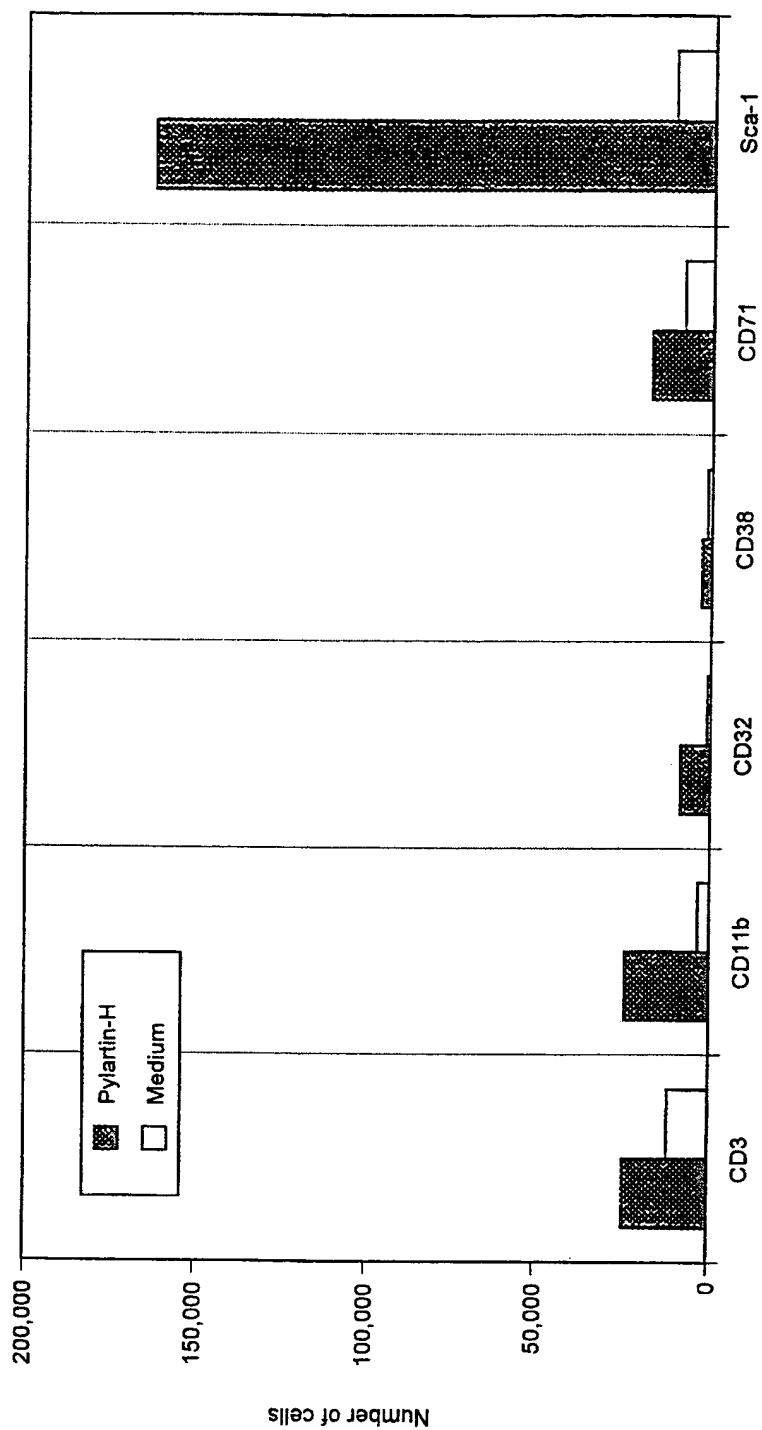
Figure 4





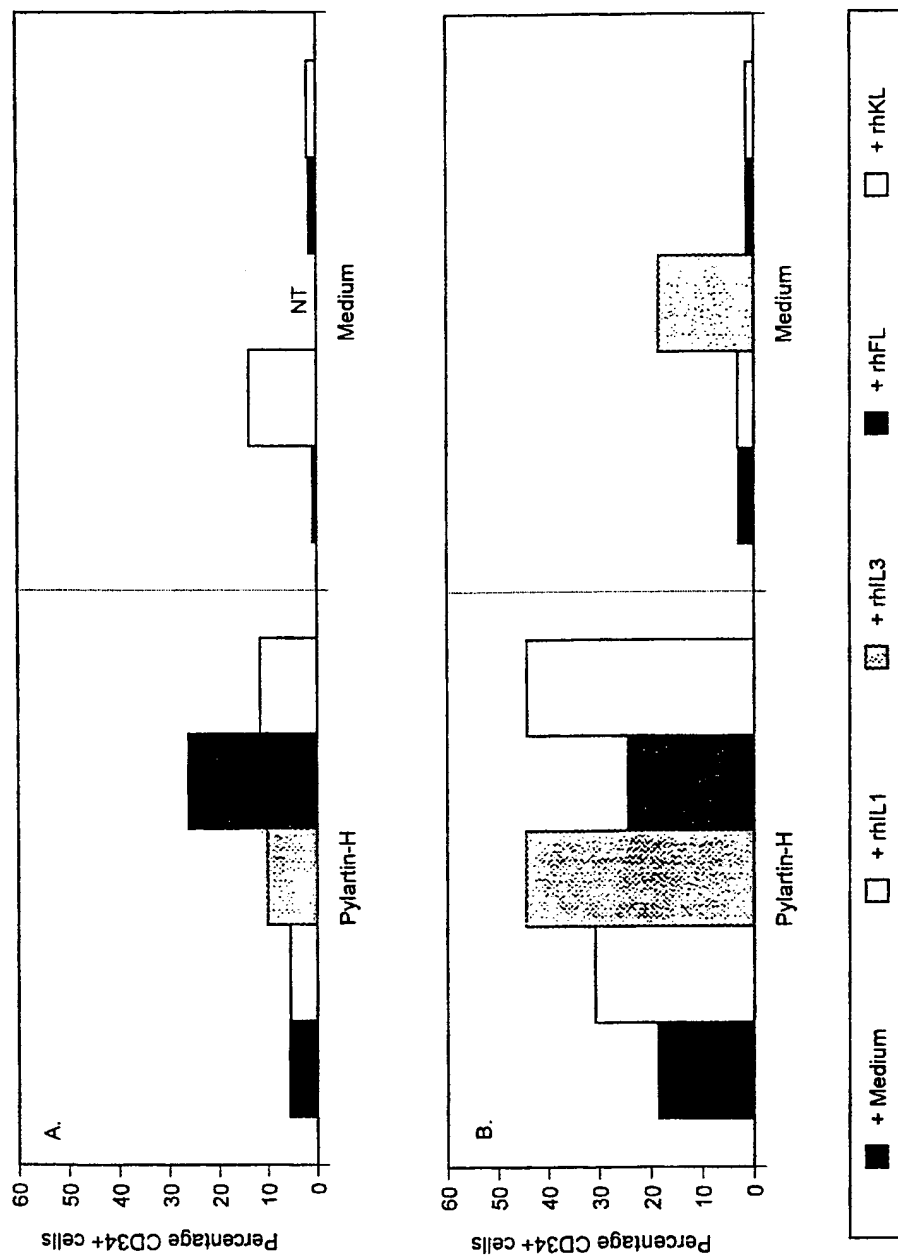
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Figure 5



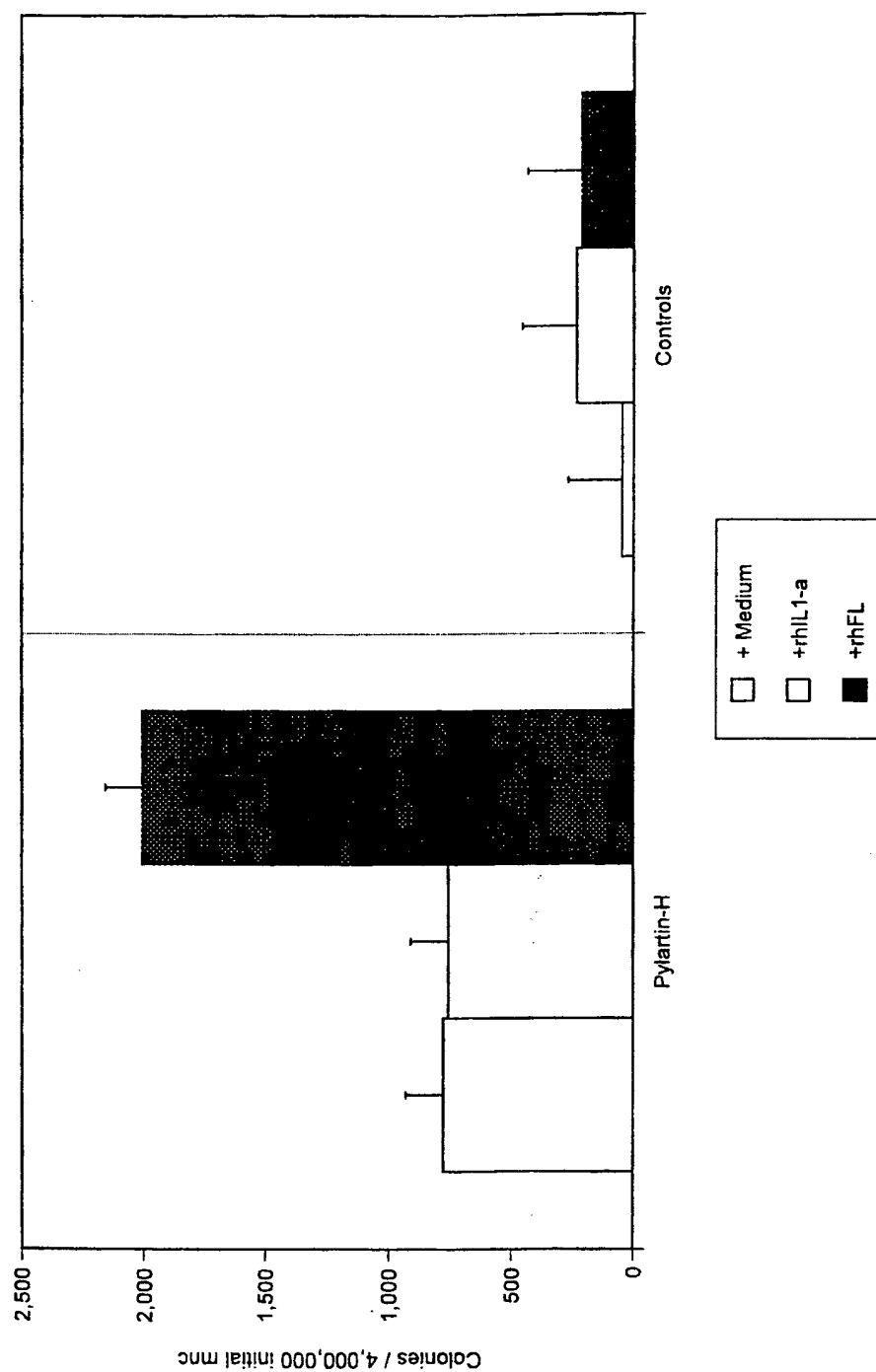
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Figures 6A - 6B



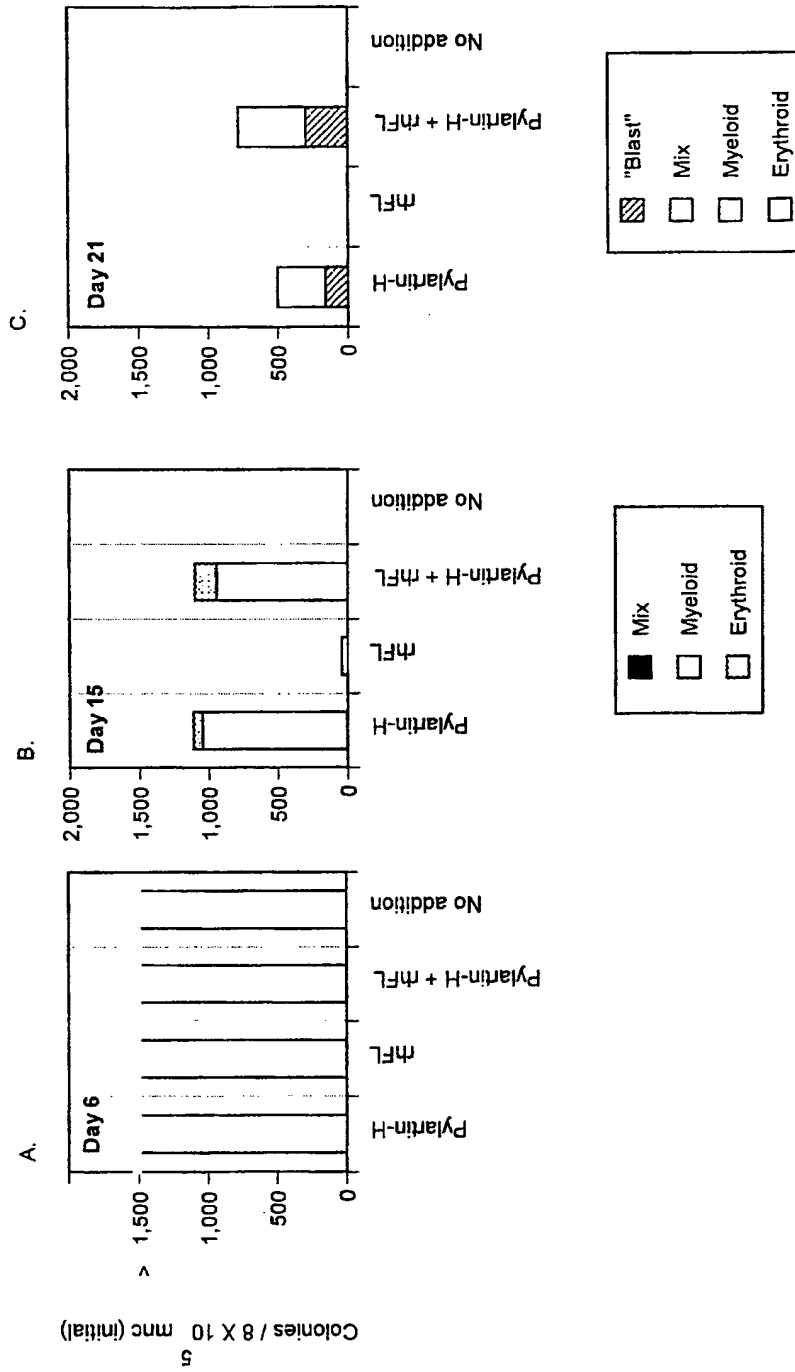
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Figure 7



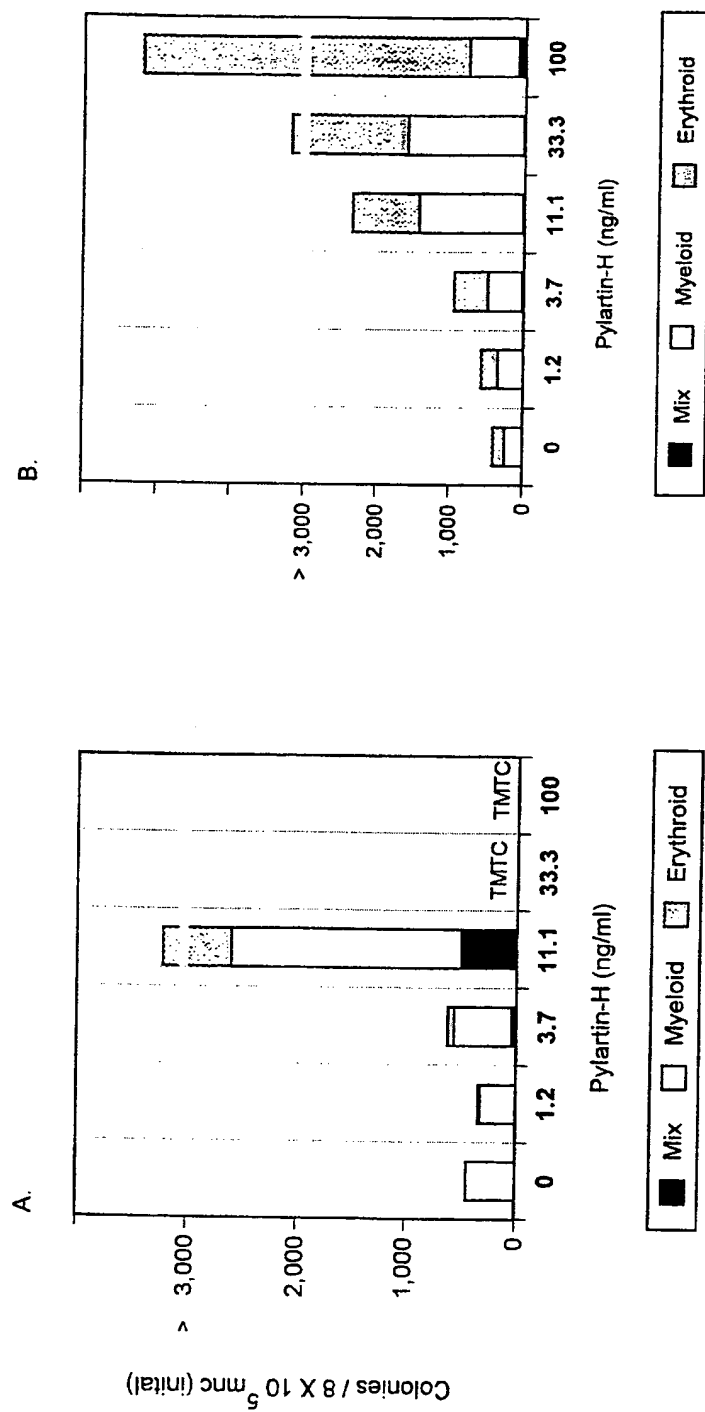
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Figures 8A - 8C



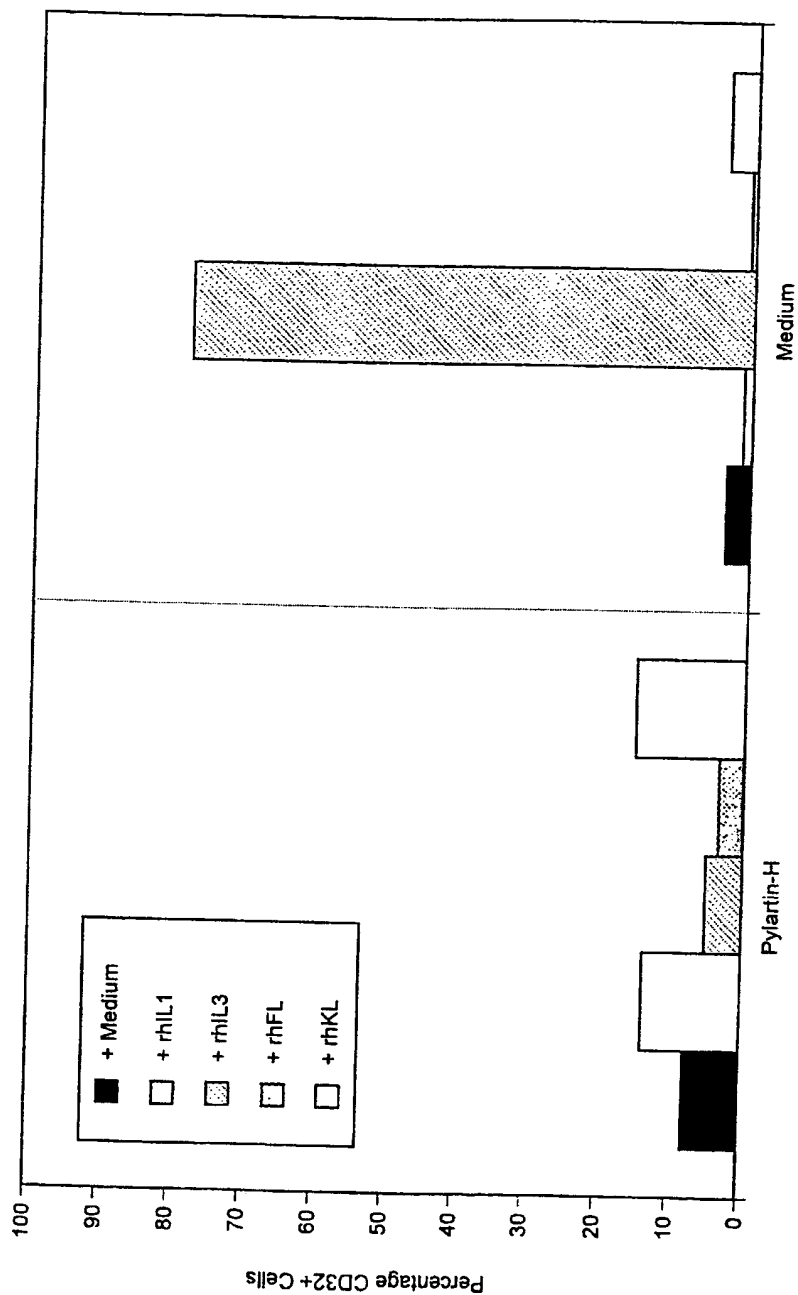
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Figures 9A - 9B



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Figure 10



## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US97/22486

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A01N 37/18

US CL : 435/325; 530/200; 514/2

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/325; 530/200; 514/2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
aps, biosis

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MORENO et al. A lectin gene encodes the alpha-amylase inhibitor of the common bean. Proc. Nat. Acad. Sci. October 1989. Vol. 86. pages 7885-7889. see entire document.	1-47

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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*P* document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

10 FEBRUARY 1998

Date of mailing of the international search report

17 MAR 1998

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